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Protein Induced by Bacteriophage T4 Which Is Absent in *Escherichia coli* Infected with Nuclear Disruption-Deficient Phage Mutants

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A protein induced by wild-type T4 phage which is absent in *Escherichia coli* infected with nuclear disruption-deficient phage (with mutations in gene *ndd*) was identified by polyacrylamide gel electrophoresis. This protein was synthesized at maximum rate at 3 to 6 min after infection. It had a molecular weight of 15,000 determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. It was associated with sedimentable fractions of the cell from which it can be dissociated with 1 M guanidine-hydrochloride. The dissociated protein can be partly recovered in a form soluble in dilute buffer after partial purification and dialysis. The occurrence of this protein in a particulate cell fraction is of interest because of the postulated role of the bacterial cell membrane in nuclear disruption.

The arrest of host gene expression and the destruction of the host DNA are dramatic events which occur shortly after infection of *Escherichia coli* by the virulent T-even bacteriophages. Certain distinct but temporally overlapping events or processes, which interact to produce the overall pattern of destruction of the host genome, have been partially resolved by cytological, genetic, and biochemical approaches. These include (i) termination of host DNA synthesis, transcription, and translation (9, 14, 20, 22), (ii) unfolding of the host chromosomes (loss of the RNA-maintained, negatively supercoiled loops or domains) (35), (iii) nuclear disruption (movement of the host DNA into juxtaposition with the cell membrane) (6, 16, 21, 23, 32), and (iv) degradation of the host DNA and incorporation of the resulting mononucleotides into phage DNA (14, 17-19, 36).

Nuclear disruption occurs during the first 2 to 3 min after infection of *E. coli* with bacteriophage T4. Phage mutants unable to induce degradation of host DNA nevertheless induce normal nuclear disruption (32). Nuclear disruption-deficient (*ndd*) T4 phage mutants have been isolated and characterized (29, 30). The complete termination of host DNA synthesis is delayed until about 10 min after infection with these mutants (28); however, unfolding of the host nucleoid (35) and shutoff of the host RNA (27) and protein synthesis (28) occur normally in cells infected with nuclear disruption and host DNA degradation-deficient multiple mutants. Unfolding of the host nucleoid is delayed in cells infected with another class of T4 phage mutants (*unf* mutants) (31). Shutoff of host DNA transcription is delayed only about 2 min, or in some instances not at all, after infection with phage bearing mutations in the *unf* gene alone or in combination with mutations in the *ndd* gene and genes inducing degradation of host DNA (34). Curiously, an active *unf* gene product is not necessary for nuclear disruption to occur (31).

We wish to report identification of a protein present in cells infected with wild-type T4 phage but absent in cells infected with nuclear disruption-deficient (*ndd*) T4 mutants. This protein has a molecular weight consistent with the coding capacity of the *ndd* gene. During isolation, it is found to be associated with particulate fractions of the bacterial cell extract.

MATERIALS AND METHODS

Phage and bacterial strains. *E. coli* strains B/5 and CR63 and wild-type phage T4D were from the California Institute of Technology collection. The nuclear disruption-deficient phage mutants *ndd*98×5 and *ndd*44×1 were prepared by backcrossing the original mutant isolates five times and one time, respectively, with T4D (29). The phage deletion mutants *r*PT8×2 and *r*1272×2 (backcrossed into a T4D background) were prepared from *r*PT8 and *r*1272 (T4B derivatives supplied by S. Champe) (28). The deletion mutants *sa*Δ3-*pset*1-*ts*A80, *sa*Δ4-*pset*1-*ts*A80, and *sa*Δ5-*pset*1-*ts*A80 and the parental strain *stp-pset*1-*ts*A80 from which they were derived were supplied by N. Cozzarelli (10). The phage mutant *unf*39×5 induces delayed

unfolding of the host nucleoid but is nuclear disruption proficient (31). The phage mutant IP°, which lacks all three viral internal proteins (4), was obtained from Lindsay Black.

Bacterial media. H broth (33) was used for growth of bacterial indicator cultures and phage stocks. EHA bottom and top agar (33) were used for phage titration from dilutions made into T broth (11). Fraser medium (13) containing 10 g of glycerol per liter and 5 g of casein hydrolysate per liter was used for growth of mass lysates of phage. M-9 medium (1) was used to grow cells for the preparation of ^{35}S-labeled extracts.

Reagents. Bacteriological media were from Difco, except casein hydrolysate which was from Nutritional Biochemicals. Urea (Schwarz/Mann ultrapure) was prepared as a saturated solution which was further purified by passage through an Amberlite MB-3 mixed-bed ion-exchange resin column. Guanidine-hydrochloride was from Aldrich Chemical Co. Sodium dodecyl sulfate from Fischer Scientific Co was recrystallized from 95% ethanol. Triton DF-16 was a gift from Rohm and Haas. Acrylamide (Eastman practical grade) was recrystallized from chloroform (100 g/liter). *N,N'*-methylenebisacrylamide (Eastman Organic Chemicals Division, Eastman Kodak Co., Rochester, N. Y.) was recrystallized from acetone (10 g/liter). *N,N,N',N'*-tetramethylethylenediamine was from Eastman Organic Chemicals. $H_2{}^{35}SO_4$ was from New England Nuclear Corp.

Preparation of ^{35}S-labeled cells. *E. coli* B/5 cells were grown in M-9 medium at 37°C with aeration by shaking to 4×10^8 cells per ml, centrifuged for 10 min at 10,000 × *g*, and washed twice with and then resuspended in M-9 medium containing $MgCl_2$ (1 mM) instead of $MgSO_4$. The cultures were adjusted to 4×10^8 cells per ml and infected with phage suspended in M-9 medium containing $MgCl_2$ instead of $MgSO_4$. The input multiplicity was 5 to 7 phage per bacterium. The cold culture of infected cells was immediately added to flasks shaking on a 30°C bath. After a specified time, $H_2{}^{35}SO_4$ (2 mCi/ml) was added to give 20 μCi/ml. To terminate labeling, the culture was made 40 mM with respect to $(NH_4)_2SO_4$. Samples were removed immediately and assayed for unadsorbed phage and unkilled bacteria (usually less than 5% unadsorbed phage, 1 to 2.5% unkilled bacteria). The cultures were chilled and harvested by centrifugation 12 min after infection.

Urea-acetic acid gel electrophoresis. An acidic polyacrylamide gel system, in which the proteins migrate as cations, was adapted from a procedure by Alfageme et al. (2). Their procedure with a gel medium containing 6 M urea-5% acetic acid-0.38% Triton DF-16 was followed explicitly except that the gels were cast as slabs 1.5 mm thick, 14 cm wide, and 15 cm long. Labeled cells were suspended in gel sample buffer, frozen and thawed three times, and centrifuged, and a sample of the supernatant solution was applied to the gel. After electrophoresis, the gels were stained with amido black (0.4%) in a water-methanol-acetic acid solution (16:5:1), destained with a water-methanol-acetic acid solution (5:5:1), and processed for detection of ^{35}S-labeled proteins by scintillation autoradiography (7).

Sodium dodecyl sulfate gel electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed as described by Castillo et al. (8) except that the gel gradient was 32 to 40% urea, 6.6 to 11% acrylamide, and 0.4 to 0.6% bisacrylamide (a modification of their system b). The samples for these gels were protein fractions from urea-acetic acid gels. They were obtained from small areas (about 3 by 25 mm) cut from the dried gels, suspended in 0.7 ml of sodium dodecyl sulfate gel sample buffer plus 35 μl of Tris, and eluted at 5°C for 7 days. Samples of the eluates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After electrophoresis, the gels were stained with amido black and prepared for scintillation autoradiography as described above for urea-acetic acid gels.

Subcellular fractionation. Two procedures were tried for subcellular fractionation. The first was procedure 1 of Ennis and Kievitt (12) in which particulate and non-sedimentable fractions of labeled infected cells are prepared in 100 mM phosphate buffer.

To investigate subcellular distribution of labeled proteins in greater detail, centrifugal fractionation was tried with solutions known to maintain 70S ribosomal structure (5, 24). This procedure, outlined in Fig. 1, yielded two particulate subfractions with different compositions, a ribosomal fraction, and a non-sedimentable fraction.

RESULTS

Identification of *ndd* protein. Phage mutants with deletions approaching or intruding into either end of the *ndd* gene have previously been isolated and mapped (Fig. 2). Separation of labeled extracts by urea-acetic acid polyacrylamide gels discloses a protein which is prominently labeled with $^{35}SO_4{}^{-2}$ shortly after infection and is absent in cells infected with phage with portions of the *ndd* gene deleted. As shown in Fig. 3, deletion *r*1272, originating in *r*II and not entering the *ndd* gene, induces the protein, whereas *r*PT8, which deletes a portion of the *ndd* gene, does not induce the protein. Likewise *sa*Δ3, which originates in *ac* and terminates before reaching the *ndd* gene, induces the protein, whereas *sa*Δ5, which enters the *ndd* gene, and *sa*Δ4, which traverses it, do not induce the protein. Functionally, phage bearing deletions *r*1272 and *sa*Δ3 are nuclear disruption proficient, whereas those bearing deletions *r*PT8, *sa*Δ5, and *sa*Δ4 are nuclear disruption deficient. Thus, an intact *ndd* gene is required both for induction of the protein observed on the gel and for nuclear disruption.

Tentative evidence that this protein is the gene product of the *ndd* gene is provided by the nuclear disruption-deficient point mutant *ndd*44×1. As shown in Fig. 3, this mutant induces a protein with slightly lower mobility on urea-acetic acid gels than the protein induced by wild-type phage. The difference of mobilities of these proteins has been verified by alternating

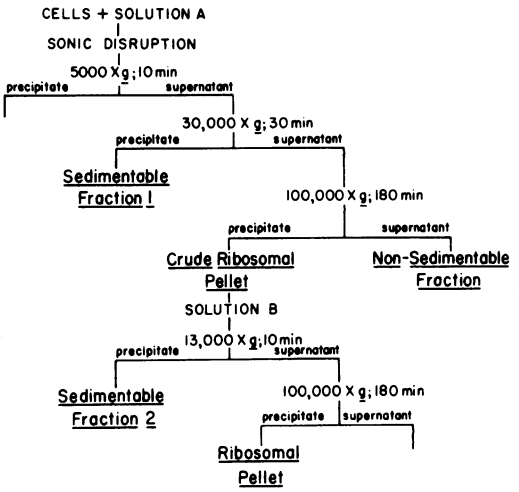

FIG. 1. *Fractionation of T4 phage-infected cells. Cells of E. coli were infected with phage T4, and the proteins synthesized after infection were labeled with* $^{35}SO_4^{-2}$ *3.5 to 6 min after infection as described in the text. Harvested cells (2 × 10*^{10} *cells) were suspended in 4.2 ml of 50 mM KCl-10 mM magnesium acetate-10 mM Tris-chloride buffer (pH 7.4)-5 mM 2-mercaptoethanol (solution A) (5) and disrupted with a Branson Sonifier, model S110 (1.5 min at setting 4). Centrifugations at 5,000 × g and 13,000 × g were in 15-ml Corex tubes with a Sorvall refrigerated centrifuge. Centrifugations at 30,000 × g and 100,000 × g were with a Beckman preparative ultracentrifuge with an SW60 rotor. The crude ribosomal pellet was suspended in 2.0 ml of 100 mM NH_4Cl-10 mM magnesium acetate-20 mM Tris-chloride buffer (pH 7.4)-5 mM 2-mercaptoethanol (solution B) (24). For gel electrophoresis, supernatant solutions were precipitated with 5% trichloroacetic acid. These precipitates and the pellets of the sedimentable fractions were dissolved in urea-acetic acid gel sample buffer (2) and, after appropriate dilutions with sample buffer, applied to the gel.*

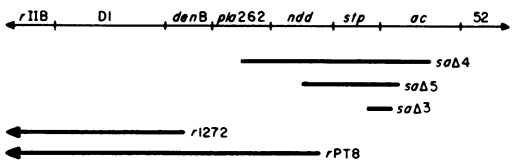

FIG. 2. *Deletion mutations investigated. These mutations, which approach or intrude into the ndd gene, were isolated and mapped by other investigators (10). The detailed genotypes of the phages bearing these mutations are described in the text.*

several samples of extracts of T4D and *ndd*44×1 on another gel (data not shown). Based on this evidence, this protein will be tentatively designated the *ndd* protein.

The *ndd* protein is not present in cultures infected with another point mutant deficient for inducing nuclear disruption, *ndd*98×5 (Fig. 3). No modified gene product for this mutant has yet been detected on urea-acetic acid or sodium dodecyl sulfate-polyacrylamide gels.

Two other nuclear disruption-proficient mutants have been examined. The mutant *unf* 39×5 is defective for unfolding of the host chromosome (31), and the mutant IP° fails to induce synthesis of three internal proteins of low molecular weight (4). Both induce normal levels of the *ndd* protein (data not shown).

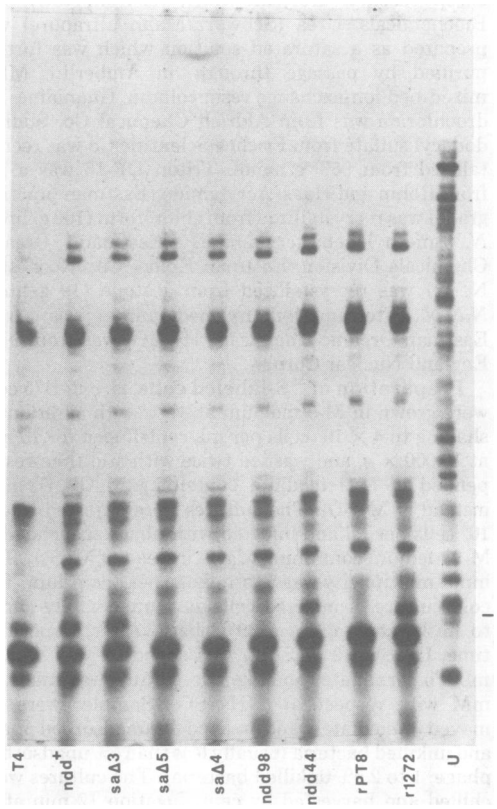

FIG. 3. *Urea-acetic acid polyacrylamide gel of whole-cell extracts of E. coli B/5 infected with strains of T4 phage. The cells were infected with phage, proteins synthesized 4 to 10 min after infection were labeled with* $^{35}SO_4^{-2}$*, and the labeled cells were collected by centrifugation and extracted with urea-acetic acid gel sample buffer (2). The material from 3 × 10*^{8} *cells was applied to each sample lane. The gel was subjected to electrophoresis for 22 h at 140 V, and the labeled proteins were visualized by scintillation autoradiography (7). Migration was downward, and the marks designate the position of the ndd protein band on the gel. The individual sample lanes are labeled: T4, T4D; ndd+, stp-pset1-tsA80 (the parental strain for the sa deletions); saΔ3, saΔ3-pset1-tsA80; saΔ5, saΔ5-pset1-tsA80; saΔ4, saΔ4-pset1-tsA80; ndd98, ndd98×5; ndd44, ndd44×1; rPT8, rPT8×2; r1272, r1272×2; U, uninfected E. coli B/5.*

Time of synthesis. A pulse-chase experiment with $^{35}SO_4^{-2}$ shows maximum rate of synthesis of the *ndd* protein 3 to 6 min after infection and no detectable synthesis after 12 min (Fig. 4).

Molecular weight. The *ndd* protein was located on a dried urea-acetic acid gel by scintillation autoradiography. The portion of the gel containing this protein was cut out and eluted, and a sample of the eluate was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

The single prominent band observed on the sodium dodecyl sulfate gel gave a molecular weight of 15,000 when compared with protein standards of known molecular weight (Fig. 5).

Bacterial protein contamination. Several prominent bacterial proteins migrate at approximately the same rate as the *ndd* protein in both urea-acetic acid and sodium dodecyl sulfate-polyacrylamide gels. One of these can be easily confused with the *ndd* protein because its rate of shutoff is slower than most bacterial proteins. This protein can be completely separated from the *ndd* protein because it migrates slightly more slowly on urea-acetic acid polyacrylamide gels, but the two proteins migrate to identical positions on sodium dodecyl sulfate gels. Although it is not prominent in some preparations in which the label is introduced at least 3 min after infection, no method of reliably excluding its labeling has been devised. Therefore, it is important to insure correct identification of the *ndd* protein, preferably with a standard known to contain both proteins to assure their adequate resolution by the urea-acetic acid gel.

Localization in particulate fractions. The *ndd* protein is associated with the sedimentable fraction of a cell extract prepared by sonication in 100 mM phosphate buffer and centrifugation at 100,000 × *g* for 18 h (12). It can be extracted from this precipitate with 1 M guanidine-hydrochloride and remains in the supernatant solution after centrifugation at 100,000 × *g* for 18 h. The slowly shut off bacterial protein mentioned above also is found in the particulate fraction and is extracted with 1 M guanidine-hydrochloride.

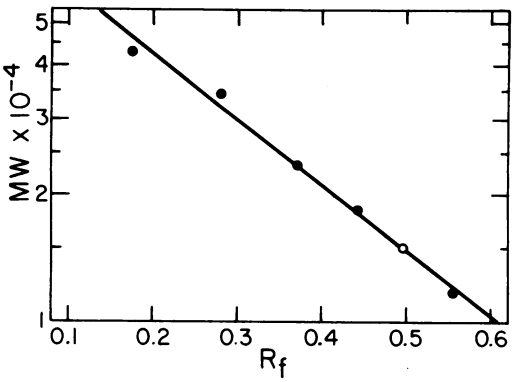

FIG. 5. *Molecular weight (MW) of ndd protein. E. coli cells were infected with T4D phage, the proteins synthesized 3 to 6 min after infection were labeled with $^{35}SO_4^{-2}$, and the labeled cells were collected by centrifugation and extracted with urea-acetic acid gel sample buffer (2). Six samples of extract, each containing material from 2 × 10^8 cells, were applied to adjacent lanes of a gel and subjected to electrophoresis for 22 h at 140 V. The ndd protein was located in the dried gel by direct autoradiography. The portion of the gel containing this band was cut out and eluted, and the eluate was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described in the text. The migration of the ndd protein on this gel, detected by scintillation autoradiography (7), was compared with the migration of standard proteins, detected by staining with amido black. Protein molecular weight standards (●): ovalbumin, 43,000; carboxypeptidase, 34,600; trypsin, 23,300; β-lactoglobulin, 18,400; cytochrome c, 11,700. The regression line was plotted by the method of least squares. The molecular weight of the ndd protein (○) is 15,000.*

To further define the particulate fraction with which the *ndd* protein is associated, cells labeled with $^{35}SO_4^{-2}$ 3.5 to 6 min after infection were subjected to the fractionation procedure diagrammed in Fig. 1. Under these conditions, the ribosomes remain as 70S units through repeated

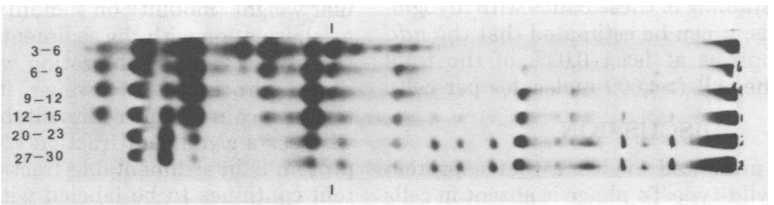

FIG. 4. *Kinetics of synthesis of ndd protein. E. coli B/5 was infected with T4D phage and pulse-labeled with $^{35}SO_4^{-2}$ at the specified times (minutes) after infection. All other procedures are identical to those described for Fig. 3, except that the gel was run for 16 h at 140 V. Migration was from right to left; the position of the ndd protein is marked.*

cycles of sedimentation and resuspension. During early steps of this fractionation, the labeled *ndd* protein is distributed in both sedimentable fraction 1 and the crude ribosomal pellet. However, when the ribosomal pellet is resuspended, the *ndd* protein remains associated with an aggregate which can easily be separated from the ribosomal fraction by low-speed centrifugation. Densitometry of scintillation autoradiograms of analytical gels shows that approximately 80% of the *ndd* protein is in sedimentable fraction 1 and 20% is in sedimentable fraction 2 (Fig. 6).

Partial purification of *ndd* protein. Although only 20% of the *ndd* protein is present in sedimentable fraction 2, it is the most heavily labeled protein in this fraction, whereas other prominently labeled proteins are found in sedimentable fraction 1 (Fig. 6). Of particular interest, little of the slowly shut off bacterial protein described above is in sedimentable fraction 2. Although the physiological basis for this fractionation has not been determined, it is reproducible and can be exploited for further purification of the *ndd* protein. Labeled sedimentable fraction 2 was extracted with 1 M guanidine-hydrochloride. The extract was clarified by centrifugation, and the supernatant solution was subjected to gel filtration chromatography (Sephadex G-75) in a solution containing 1 M guanidine-hydrochloride (Fig. 7). The peak radioactive fractions in the region of low-molecular-weight proteins were pooled and dialyzed against 20 mM Tris-chloride buffer, pH 7.4. A precipitate which formed during dialysis was collected, and it and a trichloracetic acid precipitate of the supernatant solution were subjected to urea-acetic polyacrylamide gel electrophoresis (Fig. 8). Radioactivity corresponding to the *ndd* protein was present in approximately equal amounts in the supernatant solution and the precipitate. Washing the precipitate with dialysis buffer failed to dissolve more *ndd* protein. In both the precipitate and supernatant solution, the location of the radioactive *ndd* protein band corresponded to a protein band staining with amido black (Fig. 8). By visually comparing the intensity of staining of these bands with myoglobin standards, it can be estimated that the *ndd* protein comprises at least 0.03% of the total protein of the cell (>4,000 molecules per cell).

DISCUSSION

We have presented evidence that a protein induced by wild-type T4 phage is absent in cells infected with nuclear disruption-deficient (*ndd*) deletion mutants. The protein is synthesized at maximum rate 3 to 6 min after infection, has a molecular weight on sodium dodecyl sulfate-polyacrylamide gels of 15,000, and is associated with particulate fractions of the cells. It is rendered soluble with 1 M guanidine-hydrochloride in which it can be partially purified by gel filtration chromatography.

This protein has many properties consistent with its possible role as the product of the *ndd* gene. Deletion mutations intruding into the *ndd* gene fail to induce it, whereas deletions of nearby genes do not interfere with its synthesis. A point mutant, *ndd*44×1, induces synthesis of a protein with slightly lower mobility on urea-acetic acid gels. A likely basis for this change is substitution of an amino acid residue with one of more negative charge. The molecular weight of the *ndd* protein of 15,000, determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, is consistent within experimental error with the size of the *ndd* gene of 0.5 kilobase pairs, determined by physical mapping (10). The time of maximum synthesis of this protein is consistent with evidence that the D-region of the T4 genome is transcribed with "pre-early" kinetics (26). (Gene *ndd*, formerly termed D2b, lies in this region.) A somewhat less compelling but interesting circumstance is that the functions of many genes in this region of the T4 chromosome seem to be related to cell membrane modification and repair (37). This correlates with the possible association of the *ndd* protein with a membrane fraction.

These experiments gave no insight into the molecular basis for the *ndd*98 point mutation. No peptide fragment or gene product with altered mobility was noted on urea-acetic acid or sodium dodecyl sulfate-polyacrylamide gels of extracts of cells infected with this mutant. This mutation is not suppressed in *E. coli* CR63 and is therefore not an amber chain-terminating mutant (29). Other suppressor-bearing hosts have not been investigated.

A bacterial protein with many properties similar to the *ndd* protein has been observed to be heavily labeled in a number of preparations of labeled infected cell proteins. Similar properties include mobility on urea-acetic acid gels, molecular weight (mobility on sodium dodecyl sulfate gels), isolation with the sedimentable fraction of the extract, and solubilization with 1 M guanidine-hydrochloride. However, it is almost entirely localized in sedimentable fraction 1, whereas a significant fraction (20%) of the *ndd* protein is in sedimentable fraction 2. This protein continues to be labeled with $^{35}SO_4^{-4}$ after most host protein synthesis has been shut off. Consistent with these observations are previous reports that membrane-associated bacterial proteins shut off more slowly than soluble proteins

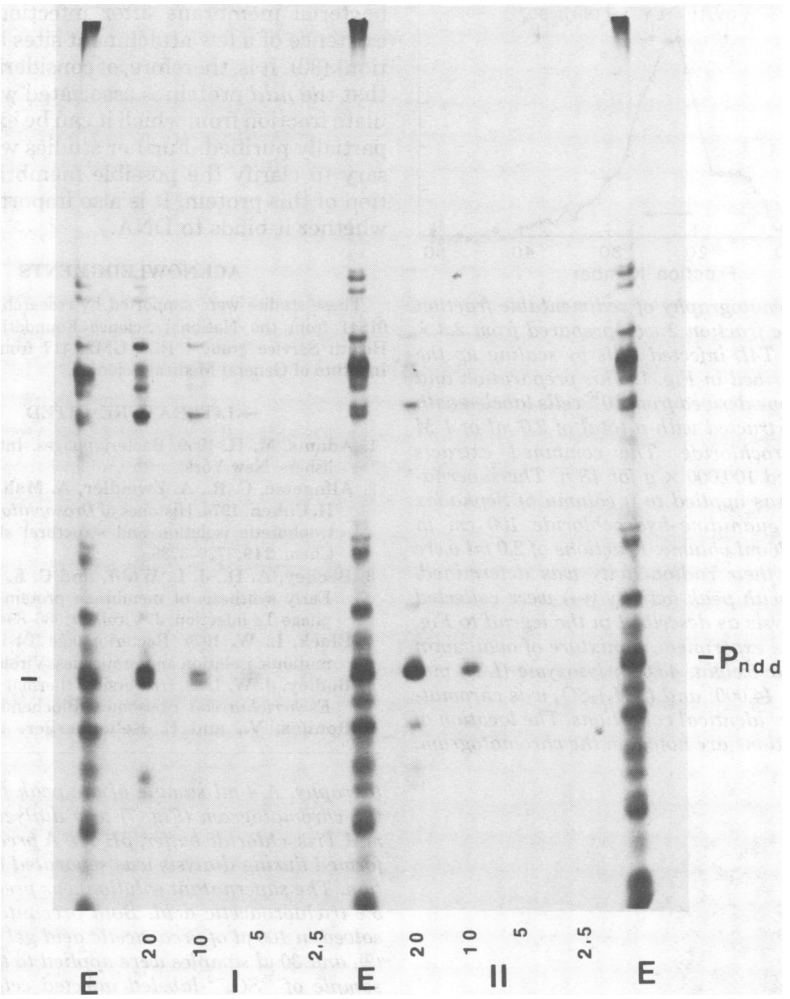

FIG. 6. *Quantitation of ndd protein in sedimentable fractions 1 and 2. E. coli cells (2×10^{10} total cells) were infected with T4D, and the proteins were labeled 3 to 6 min after infection with $^{35}SO_4^{-2}$ as described in the text. Sedimentable fractions 1 and 2 were prepared as described in the legend to Fig. 1. Also, a sample of the supernatant solution after sonic disruption and centrifugation at 5,000 × g for 10 min was removed and precipitated with 5% trichloroacetic acid. The acid precipitate of the total cell protein (designated E on the photograph) and also sedimentable fraction 1 (designated I on the photograph) and sedimentable fraction 2 (designated II on the photograph) were dissolved in urea-acetic acid gel sample buffer (2) and, after appropriate dilutions, applied to the gel. The extracts of sedimentable fractions 1 and 2 were applied in various amounts, designated on the photograph as gel sample volumes (microliters). The gel samples of total cell protein (E) each represent material from 1.3×10^8 cells; 20 μl of extract of sedimentable fraction 1, 2.0×10^8 cells; and 20 μl of extract of sedimentable fraction 2, 1.2×10^9 cells. The gel was subjected to electrophoresis for 22 h at 140 V, and the labeled proteins were detected by scintillation autoradiography (7). Migration was downward. The relative intensities of the bands of ndd protein, compared by densitometry of the film, show that approximately 80% of the ndd protein was recovered in sedimentable fraction 1 and 20% in sedimentable fraction 2. Note that another prominently labeled protein migrating slightly more slowly than the ndd protein is present in sedimentable fraction 1 but absent from sedimentable fraction 2. This is the membrane-associated bacterial protein described in the text.*

after T4 phage infection (3, 15, 25). Whether this particular protein has many properties fortuitously similar to the *ndd* protein, or is structurally or functionally related to it, is unknown.

The mechanism of nuclear disruption is unknown. Cytological and physiological evidences are consistent with a mechanism by which the host DNA becomes multiply attached to the

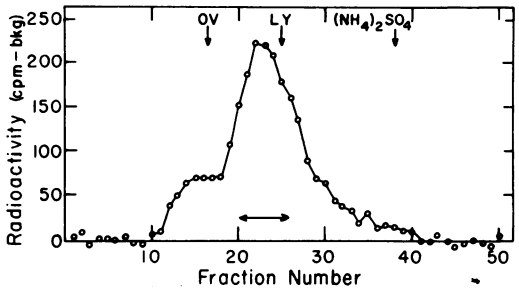

FIG. 7. *Chromatography of sedimentable fraction 2. Sedimentable fraction 2 was prepared from 2.3 × 10^{12} unlabeled T4D-infected cells by scaling up the procedure described in Fig. 1. This preparation and a comparable one derived from 10^{10} cells labeled with $^{35}SO_4^{-2}$ were extracted with a total of 2.0 ml of 1 M guanidine-hydrochloride. The combined extracts were centrifuged 100,000 × g for 18 h. The supernatant solution was applied to a column of Sephadex G-75 in 1 M guanidine-hydrochloride 100 cm in length and of 76-ml volume. Fractions of 2.0 ml were collected, and their radioactivity was determined. The fractions with peak activity (↔) were collected for futher analysis as described in the legend to Fig. 8. In a separate experiment, a mixture of ovalbumin (OV), molecular weight, 43,000; lysozyme (LY), molecular weight, 14,000; and $(NH_4)_2SO_4$ was chromatographed under identical conditions. The location of their peak fractions are noted on the chromatogram.*

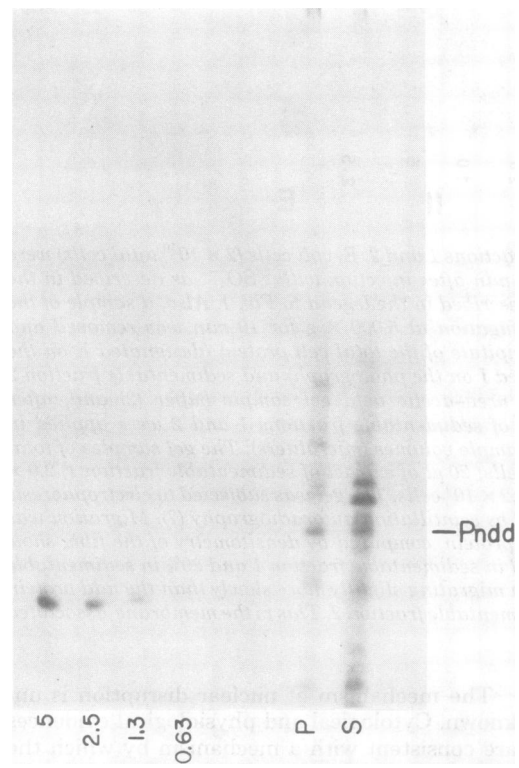

FIG. 8. *Urea-acetic acid polyacrylamide gel electrophoresis of proteins from Sephadex G-75 chromatography. A 4-ml sample of the peak fractions from the chromatogram (Fig. 7) was dialyzed against 20 mM Tris-chloride buffer, pH 7.4. A precipitate which formed during dialysis was separated by centrifugation. The supernatant solution was precipitated with 5% trichloroacetic acid. Both precipitates were dissolved in 100 μl of urea-acetic acid gel sample buffer (2), and 30-μl samples were applied to the gel. Also a sample of $^{35}SO_4^{-2}$-labeled infected cell protein was applied to verify the position of the ndd protein and different amounts of myoglobin were applied to quantitate total protein. The gel was subjected to electrophoresis for 22 h at 140 V, stained with amido black, processed for scintillation autoradiography, and dried. The photograph shows the dried stained gel. Migration was downward. The symbol Pndd designates the position of the ndd protein located by scintillation autoradiography of the labeled infected cell standard (the protein in this standard was of insufficient amount to be visualized by staining). The precipitate (P) and supernatant (S) from the dialyzed Sephadex G-75 fraction also showed radioactive bands at the position of the ndd protein. The stained protein bands which can be seen at this location on the photograph coincide with the radioactive bands on the autoradiogram. By visually comparing the intensity of these stained bands with the myoglobin standards (5, 2.5, 1.3, and 0.63 μg of protein), it can be estimated that lanes P and S each contain approximately 2 μg of ndd protein. By assuming 20% recovery of ndd protein in sedimentable fraction 2 (Fig. 6), and no other losses during fractionation, it can be estimated that the ndd protein comprises at least 0.03% of the total cell protein (>4,000 molecules per cell).*

bacterial membrane after infection (assuming existence of a few attachment sites before infection) (30). It is, therefore, of considerable interest that the *ndd* protein is associated with a particulate fraction from which it can be extracted and partially purified. Further studies will be necessary to clarify the possible membrane association of this protein. It is also important to learn whether it binds to DNA.

ACKNOWLEDGMENTS

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